

What is claimed is:

1 1. A method for detecting nucleic acid target sequences in a sample
2 comprising:
3 contacting a sample of nucleic acids with an oligonucleotide probe
4 under conditions favorable for hybridization, the oligonucleotide probe having
5 a sequence at least partially complementary to a target nucleic acid sequence
6 to be detected, the oligonucleotide probe including a fluorescent reporter
7 molecule and a quencher molecule capable of quenching the fluorescence of
8 said reporter molecule, said oligonucleotide probe existing in at least one
9 single-stranded conformation when unhybridized where said quencher
10 molecule quenches the fluorescence of said reporter molecule, said
11 oligonucleotide probe existing in at least one conformation when hybridized to
12 said target polynucleotide where the fluorescence intensity of said reporter
13 molecule when said oligonucleotide probe is hybridized to said target
14 polynucleotide is greater than the fluorescence intensity of said reporter
15 molecule when said oligonucleotide probe is not hybridized to said target
16 polynucleotide;
17 and
18 monitoring the fluorescence of said reporter molecule, an increase in the
19 fluorescence intensity of the reporter molecule indicating the presence of the
20 target sequence.

1 2. The method according to claim 1 wherein the fluorescence
2 intensity of said reporter molecule when said oligonucleotide probe is hybridized
3 to said target polynucleotide is at least about a factor of 6 greater than the
4 fluorescence intensity of said said reporter molecule when said oligonucleotide
5 probe is not hybridized to said target polynucleotide.

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1 said oligonucleotide probe existing in at least one single-stranded
2 conformation when unhybridized where said quencher molecule quenches
3 the fluorescence of said reporter molecule, said oligonucleotide probe existing
4 in at least one conformation when hybridized to said target polynucleotide
5 where the fluorescence of said reporter molecule is unquenched, the ratio of
6 the fluorescence intensities of said reporter molecule to said quencher
7 molecule when said probe is hybridized to said target polynucleotide is
8 greater than the ratio of the fluorescence intensities of said reporter molecule
9 to said quencher molecule when said probe is single-stranded;
10 and
11 monitoring the fluorescence of said reporter molecule, an increase in the
12 fluorescence intensity of the reporter molecule indicating the presence of the
13 target sequence.

1 ~~2~~ 40. The method according to claim ~~39~~ wherein the the ratio of the
2 fluorescence intensities of said reporter molecule to said quencher molecule
3 when said probe is hybridized to said target polynucleotide is at least about a
4 factor of 6 greater than the ratio of the fluorescence intensities of said reporter
5 molecule to said quencher molecule when said probe is single-stranded.

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1 12. The method according to claim 1 wherein the quencher molecule is
2 attached to a 5' terminal nucleotide of the probe.

1 13. The method according to claim 1 wherein said nucleic acid
2 polymerase is a thermostable nucleic acid polymerase.

1 14. The method according to claim 1 wherein said reporter molecule is
2 a fluorescein dye and said quencher molecule is a rhodamine dye.

1 15. A method for detecting nucleic acid target sequences in a sample
2 comprising:

3 contacting a sample of nucleic acids with an oligonucleotide probe
4 under conditions favorable for hybridization, the oligonucleotide probe having
5 a sequence at least partially complementary to a target nucleic acid sequence
6 to be detected, the oligonucleotide probe including a fluorescent reporter
7 molecule and a fluorescent quencher molecule capable of quenching the
8 fluorescence of said reporter molecule, said oligonucleotide probe existing in
9 at least one single-stranded conformation when unhybridized where said
10 quencher molecule quenches the fluorescence of said reporter molecule, said
11 oligonucleotide probe existing in at least one conformation when hybridized to
12 said target polynucleotide where the fluorescence of said reporter molecule is
13 unquenched, the fluorescence intensity of said reporter molecule being
14 greater than the fluorescence intensity of said quencher molecule when said
15 probe is hybridized to said target polynucleotide;

16 and

17 monitoring the fluorescence of said reporter molecule, an increase in the
18 fluorescence intensity of the reporter molecule indicating the presence of the
19 target sequence.

1 when said probe is hybridized to said target polynucleotide is at least about a
2 factor of 6 greater than the ratio of the fluorescence intensities of said reporter
3 molecule to said quencher molecule when said probe is single-stranded.

1 19. A method for detecting nucleic acid target sequences in a sample
2 comprising:

3 contacting a sample of nucleic acids with an oligonucleotide probe
4 attached to a solid support under conditions favorable for hybridization, the
5 oligonucleotide probe having a sequence at least partially complementary to a
6 target nucleic acid sequence to be detected, the oligonucleotide probe
7 including a fluorescent reporter molecule and a quencher molecule capable of
8 quenching the fluorescence of said reporter molecule, said oligonucleotide
9 probe existing in at least one single-stranded conformation when
10 unhybridized where said quencher molecule quenches the fluorescence of
11 said reporter molecule, said oligonucleotide probe existing in at least one
12 conformation when hybridized to said target polynucleotide where the
13 fluorescence intensity of said reporter molecule when said oligonucleotide
14 probe is hybridized to said target polynucleotide is greater than the
15 fluorescence intensity of said reporter molecule when said oligonucleotide
16 probe is not hybridized to said target polynucleotide;

17 and

18 monitoring the fluorescence of said reporter molecule, an increase in the
19 fluorescence intensity of the reporter molecule indicating the presence of the
20 target sequence.

1 20. The method according to claim 19 wherein the fluorescence
2 intensity of said reporter molecule when said oligonucleotide probe is hybridized
3 to said target polynucleotide is at least about a factor of 6 greater than the

